



Effect of terminal locations of pods on biomass production and ^{13}C partitioning in a fasciated stem soybean Shakujo

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Abstract: This study addresses the hypothesis that stagnation of soybean yield on the farm can be improved by selection of a physiological trait favoring carbon assimilate partitioning to terminally placed pods versus genotypes having axillary pods at close plant spacing. ^{13}C was fed to source-sink units comprising a leaf, axillary/terminal pods, and petioles at upper and lower positions of the stem axis in two soybean cultivars, namely Shakujo and Enrei, at different densities of populations. The cultivars differ significantly in architecture, Shakujo bearing a few hundreds of pods in close succession to one another in a terminally placed raceme, in contrast to Enrei having axillary racemes. Pod yield per plant was higher in Enrei than in Shakujo at low density, but Shakujo out-yielded Enrei at close spacing. Population density decreased yield per plant and altered the pattern of assimilate partitioning significantly within the plants for both varieties. At high density more assimilates moved to the upper parts at the cost of the lower parts. The terminally placed pods of Shakujo were advantaged to receive assimilates under density stress. No benefit was accrued to pod filling of Enrei, however, under this condition.

Key words: Fasciated stem, Grain yield, Photosynthesis, Photosynthate partitioning, Population density, Soybean, Source-sink unit

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1 Introduction

Soybean seed is a rich source of edible proteins and oil for human beings. It ranks fifth among the grain crops cultivated worldwide (Egli and Crafts-Brandner, 1996). The seed yield of this important crop has improved through cultivar development (Specht *et al.*, 1999). The rise in annual production

has ranged between 0.5% and 0.7% in North America, the world's greatest soybean-producer (Kumudini, 2002). However, to sustain growth of soybean production, a physiological understanding of yield improvement is necessary, in particular, by comparison of the improved genotypes with the traditional genotypes under various cultural practices. Similar to other grain crops, yield is a product of interaction between genotypes and the environment (Liu and Herbert, 2002); the most suitable combination is desired for maximizing grain yield. In this context, the response

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of soybean genotypes to planting density has attracted considerable attention (Egli, 1988; Bullock *et al.*, 1998; Gan *et al.*, 2002). The spatial distribution of soybean plants has been reported to influence growth and yield responses (Duncan, 1986; Robinson and Wilcox, 1998) by affecting canopy photosynthesis and light interception capacities (Wells, 1991; Board, 2000; Singer, 2001). Close spacing enhances light interception. The canopy photosynthesis increases and supports greater seed yield (Christy and Porter, 1982; Board *et al.*, 1992; Singer, 2001).

A positive correlation of seed yield with population density is not always evident in soybean. There have been instances in which seed yield did not increase commensurate to the increase of population density (Weaver *et al.*, 1991; Kane and Grabau, 1992; Board, 2000). Among the genotypes which respond positively to population density, the effect of plant density on seed yield varies independent of genotype (Gan *et al.*, 2002). This evidence indicates differences in physiological interactions among the genotypes in response to population density. The chemical nature of these responses is thus far elusive.

Seed yield of grain crops is a direct outcome of biomass partitioned from the source to the sink, but there have been fewer studies in soybean to unravel the influence of population density on this aspect and to understand how genotypes differ in response to plant density.

In the present study, biomass partitioning has been examined in a fasciated stem soybean, Shakujo, in which the architecture is modified to shift the spatial locations of the sources and sinks, although this genotype has not been intensively studied in terms of biomass and grain production (Albertsen *et al.*, 1983). It seems that the peculiar architecture of the genotype precludes mutual shading under constraints of population density stress and is congenial for easy flow of assimilates to sink from the source, in comparison with common cultivars of Japan. In the process here, a concept will be developed for the plant ideotype for better partitioning of assimilates for high grain yield.

2 Materials and methods

Two soybean (*Lysine max* L.) genotypes, namely a fasciated stem (f/f) soybean Shakujo and a normal

stem (F/F) soybean Enrei were used in the experiment. Shakujo originated in Japan in the course of natural mutation. Expression of a recessive gene is responsible for the swelling of the apical internodes. This genotype bears a few hundred of pods in close succession to one another on the flowering raceme located at the top of the stem. The leaves are found in whorls, according to the verticillate type of phyllotaxy. In contrast, Enrei is a common genotype widely cultivated in Japan. On the axillary position of many leaves, a flowering axis containing a raceme type of inflorescence is borne. Each raceme consists of a maximum number of three pods. The leaves are arranged in alternate sequence on the stem and the order of development of pods is acropetal.

2.1 Experiment 1: ^{13}C export and partitioning from leaves at different positions on the main stem

Shakujo and Enrei cultivars were cultivated in the experimental field of Hiroshima University from May to October in 2002 and 2003. The soil was a granite aerosol. Commercial fertilizers consisting of N, P, and K were added to the soil at the ratio of 50:98:139 kg/ha. Soil pH was adjusted to 6.0 with dolomitic calcium carbonate prior to a basal dressing. Three seeds were planted in a hill spaced at 40 cm apart in a row and the rows were spaced 70 cm apart, equivalent to 3.7 plants/m². There were 40 rows in an individual plot. The plants in a hill were thinned to one plant 21 d after cultivation. The statistical design was a randomized block with three replicates.

2.1.1 $^{13}\text{CO}_2$ feeding

A trifoliated leaf at different positions of the stem was exposed to $^{13}\text{CO}_2$ 14 d after flowering according to the method described by Nobuyasu *et al.* (2003). The leaf was placed inside a transparent plastic bag containing 1 g of Ba¹³CO₃ (atomic fraction 99% ^{13}C). A total of 4 ml lactic acid was injected into the bag to release the $^{13}\text{CO}_2$. The leaf was allowed to assimilate $^{13}\text{CO}_2$ for 2 h under average photosynthetically active radiation (PAR) of 1000 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$. The plants were harvested immediately after termination of $^{13}\text{CO}_2$ assimilation. Harvested plants were immediately separated into the ^{13}C fed leaf, ^{13}C fed leaf petiole, pods of the same unit with ^{13}C fed leaf (in Shakujo, it is referred to pods at top unit), other pods, and the rest of the leaves and stem above or below the

^{13}C fed leaf, which are referred to as upper or lower leaves and stem. The plant parts were dried in an open air oven at 70 °C for 3 d, weighed and ground to powder with a vibrating sample mill (Model TI-100 Heiko Co., Ltd., Fukushima, Japan).

2.1.2 ^{13}C analysis

The ^{13}C abundance in the sample was determined with a mass spectrometer (Finnigan MAT Delta Plus) using 2 μg of the powdered materials. The amount of labeled C (A) was calculated using the equation as follows: $A = \frac{^{13}\text{C} \text{ abundance in the sample}}{^{13}\text{C} \text{ abundance of Ba}^{13}\text{CO}_3} \times \text{amount of C in the sample}$.

2.2 Experiment 2: grain weights and source activities of upper and lower units

In this experiment both of the genotypes were grown in the field conditions at hill and row distances of 20 and 50 cm, respectively. The method of cultivation was similar to Experiment 1. During the pod filling stage, the uppermost source-sink unit (referred to as upper unit) consisting of the leaf, petiole, axillary pod, and sub-tending stem was separated from the rest by girdling treatment. The fifth unit (lower unit), consisting of similar segments, was also excluded from the rest by a similar technique. Girdling was done by heating a part of the stem in a circular fashion just above and below the unit with the help of an electric burner. On the following day, ^{13}C was fed to the leaves of these two units and partitioning of the isotope was traced in different plant parts as described in Experiment 1. In addition, leaf area, photosynthetic rates of the leaves, and grain yields of the two units were also measured. There were three replicates for each observation.

Photosynthetic rate was measured by a portable infra-red gas analyzer (Model LI-6400, LI-COR Co., Ltd., Lincoln, USA) under natural sunlight. The PAR was more than 1700 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ during the measurements. The leaf area was determined by an auto leaf area meter (Model AAM-5, Hayashi Denko Co., Ltd., Osaka, Japan). The dry weights of the plant parts were measured at maturity.

2.3 Experiment 3: effects of population density on biomass, grain production, and ^{13}C partitioning

Both genotypes were grown in the field condi-

tions similar to Experiment 1 at four levels of population densities: 6.5, 12.5, 18.0, and 22.9 plants/ m^2 . The experiment was conducted in a randomized block design with three replicates.

The production structure at 106 d after treatment (DAT) was investigated. The light transmission ratio (LTR), leaf area, and pod weight at the different strata were measured. LTR was investigated by measurement of solar radiation intensity at the various heights of the canopy with a portable infra-red gas analyzer (Model LI-6400, LI-COR Co., Ltd., Lincoln, USA). The photosynthetic rates of the upper and lower strata at various radiation levels were measured.

The $^{13}\text{CO}_2$ feeding, as described in Experiment 1, was carried out using leaves at the upper (the first) and lower (the fifth) units counted from the uppermost leaf in different densities of population during the pod filling stages.

2.4 Statistical analysis

All the experiments were analyzed for the effect of treatments according to the expected mean squares given by McIntosh (1983). For the separation, treatment sum of squares was partitioned by the method of orthogonal contrasts. The coefficient of variation for all response variables was below 10%.

3 Results

3.1 Experiment 1: ^{13}C export and partitioning from leaves at different positions on the main stem

3.1.1 Biomass production

Biomass production similarly increased with time in both soybean cultivars, Shakujo and Enrei (data not shown). This increase in pods initiated earlier in the former than in the latter.

3.1.2 ^{13}C export and partitioning

In Shakujo, ^{13}C partitioning to the apical pods was maximum from the uppermost leaf. The contributions from leaf Nos. 3 and 5 were also significant (Fig. 1). However, leaf No. 7 did not contribute any of the ^{13}C fed to it for development of the apical pods. Instead, some of ^{13}C from this leaf moved into the axillary pods. Leaf Nos. 3 and 5 contributed more ^{13}C

to axillary pods in comparison with the apical pods. Leaf No. 1 retained nearly 60% of the ^{13}C fed, but the rate of transport was higher in leaf Nos. 3 and 5 within the same period. In contrast, transport of ^{13}C was the poorest in leaf No. 7; it retained about 80% of assimilates.

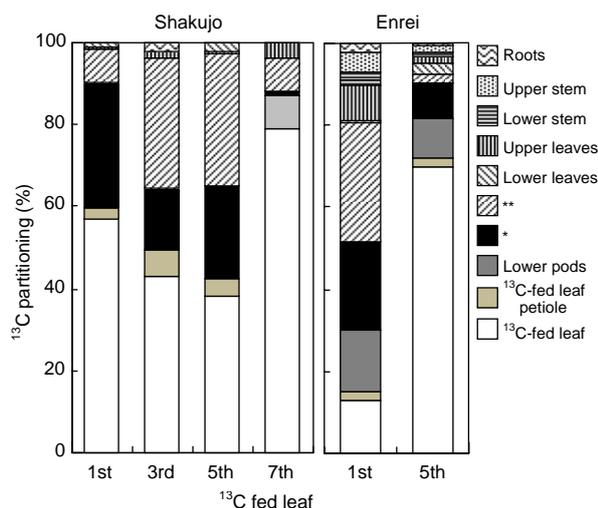


Fig. 1 Partitioning ^{13}C fed from leaves at different positions during pod filling stage in Shakujo and Enrei. ^{13}C was fed to leaf Nos. 1, 3, 5, and 7 in Shakujo, and leaf Nos. 1 and 5 in Enrei. The leaves were counted from the uppermost leaf. * Apical pods for Shakujo and unit pods for Enrei; ** Axillary pods for Shakujo and upper pods for Enrei. The experiment was conducted in 2002

Similar to Shakujo, export of ^{13}C from an upper leaf was more than that of the lower leaf in the Enrei cultivar; the lower leaf retained nearly 80% of the fed isotope (Fig. 1). A major part of the assimilate export from the upper leaf went to the pods of the ^{13}C fed unit and the pods above it. The pods below the fed leaf received a minor share. In contrast, export of ^{13}C from the lower leaf was identical to the pods on its own unit and below.

3.2 Experiment 2: grain weights and source activities of upper and lower units

In Shakujo, the upper source-sink units possessed considerably more numbers of pods and grains compared with the lower units (Table 1). However, the average grain weight did not differ as much between the units. Unlike Shakujo, the pod and grain numbers did not differ between the upper and lower units in Enrei cultivar, but the grain weight of the upper unit was significantly higher than that of the lower unit.

The source capacity of the upper unit was considerably higher than that of the lower unit in both Shakujo and Enrei cultivars during the period of pod filling (Table 2). With passage of time, the source activity of the upper unit declined significantly, but it was not so for the lower unit. Differences in photosynthetic rate and leaf area contributed to the variation

Table 1 Grain weight and its parameters of upper and lower units in Shakujo and Enrei

Cultivar	Position	m_{grain} (g)*	n_{pod}	n_{grain}	$m_{100\text{-grain}}$ (g)
Shakujo	Upper units	29.70 (86.8%)	126.3	219.8	13.5
	Lower units	0.75 (2.1%)	4.1	6.9	11.6
Enrei	Upper units	1.68 (4.2%)	3.0	8.2	21.9
	Lower units	1.02 (2.6%)	2.8	7.7	13.0
LSD (0.05)		14.3	61.5	106.1	4.7

m_{grain} : grain weight per unit; n_{pod} : number of pods per unit; n_{grain} : number of grains per unit; $m_{100\text{-grain}}$: 100-grain weight. * Figures in the parentheses are percentages relative to whole plant grain weight. Grain yield and yield components were measured at 149 and 135 DAP in Shakujo and Enrei, respectively. LSD: least significant difference. The experiment was conducted in 2003

Table 2 Photosynthetic rate, leaf area, and source capacity of Shakujo and Enrei at the pod-filling stages

t (DAT)	Position	Shakujo			Enrei		
		PR ($\mu\text{mol}/(\text{m}^2\cdot\text{s})$)	S_{leaf} (m^2)	SC ($\mu\text{m}/\text{s}$)	PR ($\mu\text{mol}/(\text{m}^2\cdot\text{s})$)	S_{leaf} (m^2)	SC ($\mu\text{m}/\text{s}$)
101	Upper units	15.9	0.0277	0.440	16.9	0.0240	0.406
	Lower units	4.8	0.0117	0.056	12.3	0.0101	0.124
108	Upper units	10.2	0.0105	0.107	12.6	0.0255	0.321
	Lower units	2.1	0.0167	0.035	6.5	0.0196	0.127
116	Upper units	4.6	0.0187	0.086	6.1	0.0252	0.154
	Lower units	3.8	0.0110	0.042	3.7	0.0062	0.023
LSD (0.05)		5.2	0.0066	0.155	5.0	0.0083	0.142

PR: photosynthetic rate; S_{leaf} : each leaf area; SC: source capacity per leaf; LSD: least significant difference. The experiment was conducted in 2003

of source activities of the upper and lower units in both the cultivars, but the contribution of the upper unit was more compared with the lower unit. At 101 and 108 DAT, the photosynthetic rate of the upper unit of Shakujo was more than three times higher than that of the lower unit. However, this difference was not evident in the Enrei cultivar.

Girdling of the upper and lower units restricted ^{13}C partitioning within the various components of the units. In Shakujo, ^{13}C fed to the leaf in the upper unit moved to the pods and the translocation increased with the passage of time (Fig. 2). No ^{13}C moved from the leaf of the lower unit to the pods, although this leaf assimilated the isotope. The situation was different for Enrei. At 109 DAT, ^{13}C export to the pod was very high. It declined considerably at 117 DAT, and the most isotopes were retained in the fed leaf blade. The fifth leaf could assimilate very little amount of ^{13}C fed.

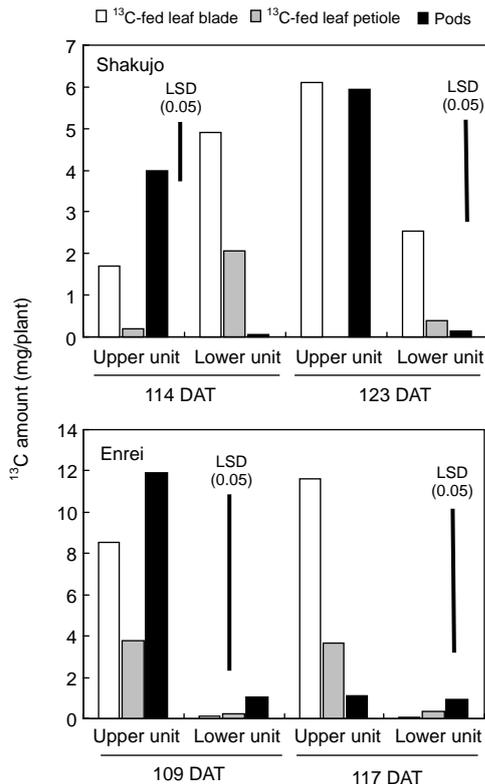


Fig. 2 Partitioning of fed ^{13}C to different plant parts in upper (the first unit from the top) and lower (the fifth unit, the lowest) source-sink units in Shakujo and Enrei. ^{13}C was fed to the leaf in each unit. The plants were girdled right below the unit. LSD: least significant difference. The experiment was conducted in 2003

3.3 Experiment 3: effects of population density on biomass, grain production, and ^{13}C partitioning

3.3.1 Grain production

Biomass production decreased with increasing density and the decrease was similar in Shakujo and Enrei (Fig. 3). Increase of population density significantly improved grain yield per plant in Shakujo. In contrast, the yield of Enrei was not influenced by the density (Table 3). At low density, Enrei produced more yield than Shakujo, but the latter out-yielded the former at high density. The yield advantage of Shakujo at high density was due to the difference in pod number per plant. Population density did not alter pod number in Shakujo, but it reduced the number significantly in Enrei. Grain weight of either of the two cultivars was not affected by population density.

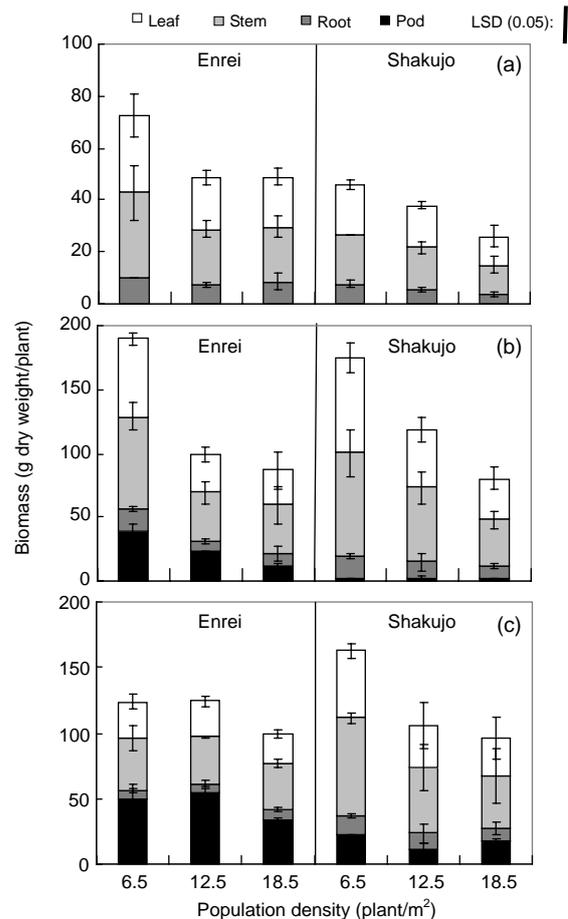


Fig. 3 Effect of population density on biomass production of soybean at different growth stages in Shakujo and Enrei

(a) 77 DAT; (b) 98 DAT; (c) 114 DAT. LSD: least significant difference. The experiment was conducted in 2003

Table 3 Grain weight and yield components in Shakujo and Enrei at different population densities

Cultivar	Population density (plant/m ²)	Grain weight		n_{pod}	n_{grain}	$m_{100\text{-seed}}$ (g)
		m_1 (g/m ²)	m_2 (g/plant)			
Shakujo	6.5	247	38.0±14.3	171.5±39.6	233.0±75.7	16.3±0.21
	12.5	441	35.3±12.2	150.6±36.1	215.2±64.0	16.4±0.35
	12.5*	499	39.9		201.5	19.8±0.35
	18.5	673	36.4±8.0	148.9±26.3	211.6±42.8	17.2±0.57
	18.5*	588	31.8		166.5	19.1±0.57
Enrei	6.5	568	87.4±12.6	173.1±27.8	284.7±42.2	30.7±0.92
	12.5	615	49.2±9.6	100.0±22.8	155.7±39.4	31.6±0.64
	12.5*	512	41.0		167.3	30.6±0.51
	18.5	594	32.1±11.7	73.8±18.5	104.3±31.2	30.8±1.06
	18.5*	669	36.1		104.3	34.6±0.33

m_1 : grain weight per unit (m²) of area; m_2 : grain weight per plant; n_{pod} : number of pods per plant; n_{grain} : number of grains per plant; $m_{100\text{-seed}}$: 100-seed weight. Grain yield and yield components in 2003, and 2004* were measured at maturity. In 2004, Shakujo plants lodged at 110 DAT. Values are expressed as mean (±SD)

3.3.2 Leaf area distribution at different strata

The total leaf area of the plant was higher in Shakujo than in Enrei (Fig. 4). Population density stress significantly reduced the leaf area in both cultivars, but it was more effective in Shakujo. The leaf area of Shakujo at the uppermost stratum remained unchanged at 50% of total area at different densities of population. For Enrei, however, the leaf area of the uppermost stratum was more than 50% at low population density, but it decreased to less than 50% at high density.

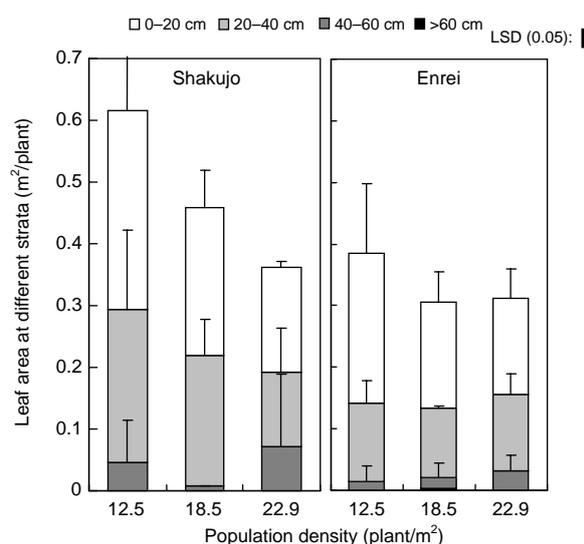


Fig. 4 Effect of population density on leaf area distribution at different strata of soybean canopies of Shakujo and Enrei at the pod filling stage (106 DAT)

Flowering, pod elongation, and grain filling initiated at 55, 77, and 102 DAT in Enrei, and 69, 91, and 115 DAT in Shakujo, respectively. LSD: least significant difference. The experiment was conducted in 2003

3.3.3 Pod mass distribution at different strata

The total pod weight per plant was nearly two times higher in Enrei than in Shakujo at low population density (Fig. 5). The weight decreased as population density increased in both cultivars. At higher densities, the pod weight of Shakujo was more depressed than Enrei; at densities of 18.5 and 22.9 plants/m², the pod weight of the former was less than a third of pod weight of the latter (Fig. 5). In Shakujo, pod weight distribution was mostly confined to the uppermost stratum of the plant, and density stress did not alter

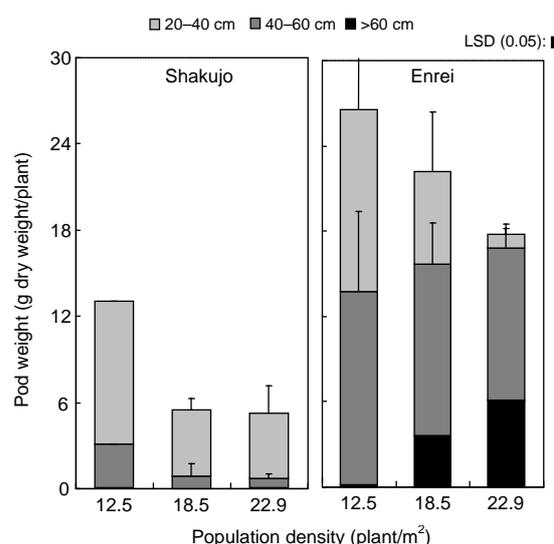


Fig. 5 Effect of population density on pod weights of Shakujo and Enrei during the pod filling stage (106 DAT)

Flowering, pod elongation, and grain filling initiated at 55, 77, and 102 DAT in Enrei, and 69, 91, and 115 DAT in Shakujo, respectively. LSD: least significant difference. The experiment was conducted in 2003

this pattern. In contrast, the distribution pattern was seriously affected by density stress in Enrei. At low density, pod weight distribution was equally divided between the strata of 20–40 and 40–60 cm. But at higher densities, distribution of pod weight in favor of the lower two strata increased at the cost of the uppermost stratum.

3.3.4 Light transmission ratio in the canopy

LTR was higher in the leaves of the upper unit than in those of the lower unit in both genotypes, which was maintained in spite of population density (Fig. 6). In contrast to this, that in the leaves of the lower unit decreased with lowering position of unit and the decrease was more intense in Shakujo compared with Enrei.

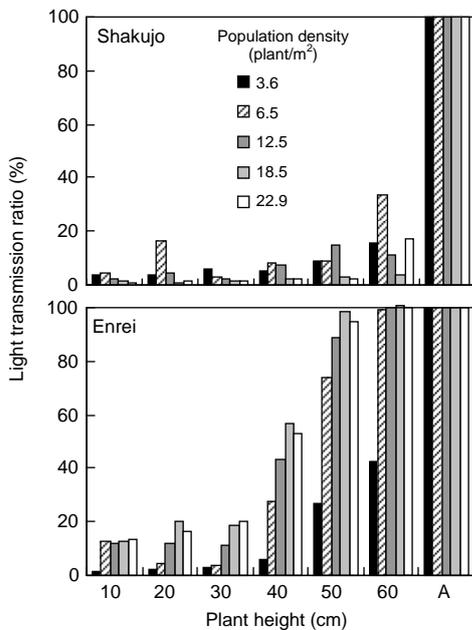


Fig. 6 Effect of population density on light distribution status of Shakujo and Enrei

A: above plant canopy (60 cm). The experiment was conducted in 2004

3.3.5 Leaf photosynthetic rate

Leaf photosynthetic rate tended to decline temporally in both the soybean cultivars during the period of observation at 75 to 101 DAT. The upper leaves in both cultivars had similar rates of leaf photosynthesis and population density rarely affected the process (Figs. 7 and 8). In both cultivars, the rate of photosynthesis was higher in the upper than in the lower leaves. The rate of photosynthesis in the lower leaves

was higher in Enrei than in Shakujo at low densities below 18.5 plants/m²; however, the difference was not observed at high density.

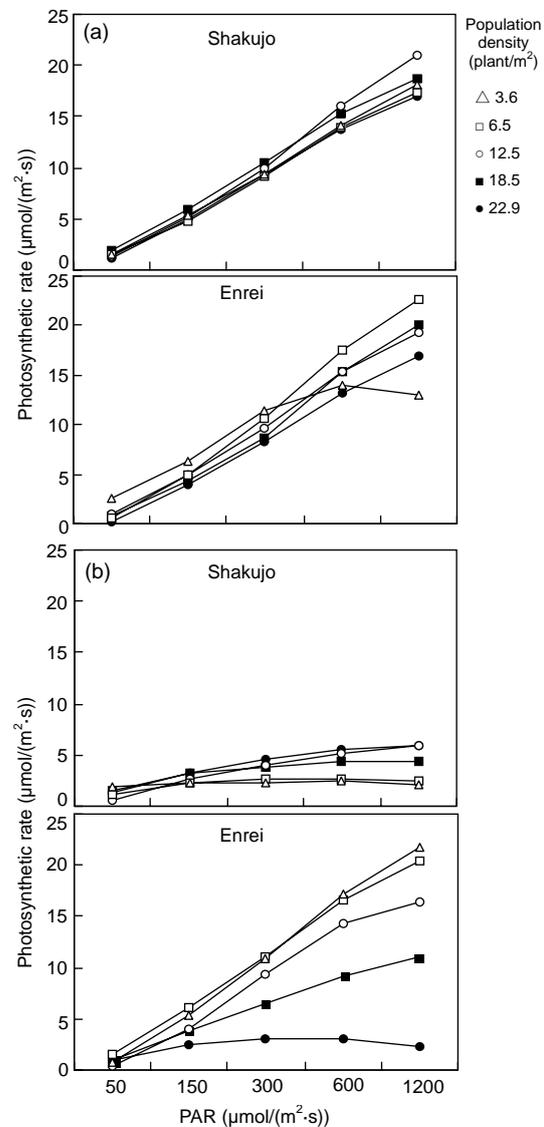


Fig. 7 Effect of photosynthetically active radiation (PAR) on photosynthetic rates of upper (a) and lower (b) leaves of Shakujo and Enrei at the different population densities

The upper and lower leaves are the 1st and 5th leaves. The experiment was conducted in 2004

3.3.6 ¹³C partitioning

In Shakujo, ¹³C fed to the leaf of the upper unit mostly accumulated in the pods of the same unit and nearby units (Fig. 9, Table 4). Isotopes fed to the leaf of the lower unit were not translocated as much as those of the upper unit. They were poorly translocated to the pods and retained mostly in the vegetative parts

like leaf or stem at low density. At high density, export from the upper unit significantly increased to the pods of its own unit and export to other pods declined severely. At high density, export to pods increased from the lower unit as well, but the increase was limited. Export of ^{13}C from upper and lower units was not

similar in Enrei. At low density, export of ^{13}C was mostly in favor of the pods from both the units. At high density, export from the upper unit increased in the pods of its own unit at the cost of the other pods. The situation was, however, the opposite for the export of ^{13}C from the lower unit.

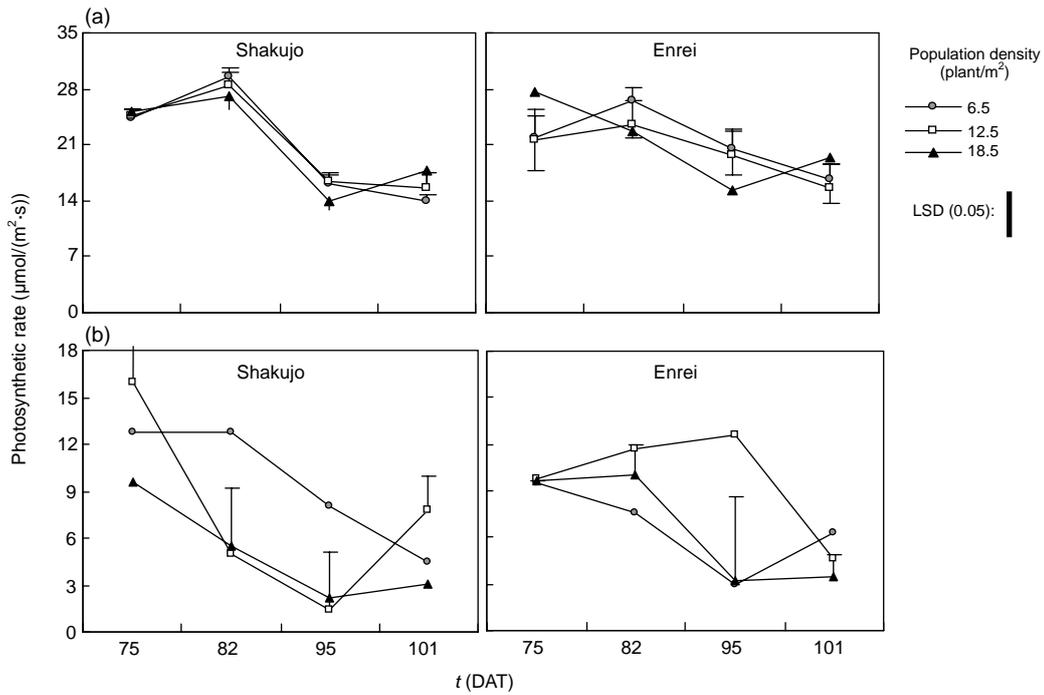


Fig. 8 Effect of growth stage on photosynthetic rates of upper (a) and lower (b) leaves of Shakujo and Enrei at different population densities

LSD: least significant difference. The experiment was conducted in 2002

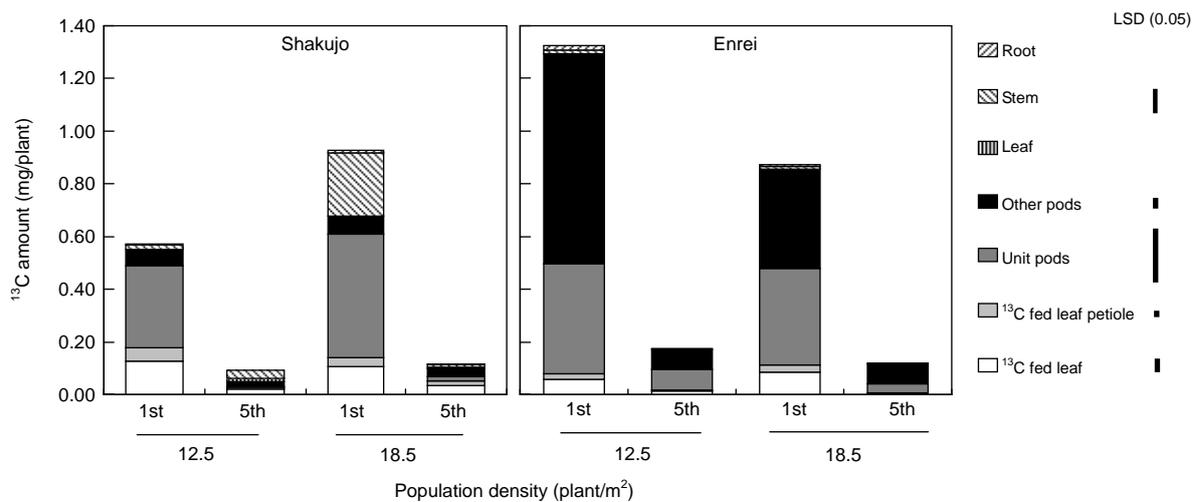


Fig. 9 Effect of population density on ^{13}C partitioning among various plant parts during pod-filling stage in Shakujo and Enrei

In Shakujo, unit pods are the pods at the top on the main culm; in Enrei, they are the pods of the unit where ^{13}C -fed leaf positioned. LSD: least significant difference. The experiment was conducted in 2003

Table 4 Effect of population density on excess atomic fraction of ^{13}C among various plant parts during pod-filling stage in Shakujo and Enrei

Population density and ^{13}C fed leaf	Plant parts	Excess atomic fraction of ^{13}C (%)	
		Shakujo	Enrei
Low density 1st unit	^{13}C fed leaf	0.261	0.057
	^{13}C fed leaf petiole	0.229	0.041
	Other leaves	0.000	0.000
	Stem	0.001	0.001
	Other pods	0.007	0.035
	Pods in an unit	0.035	0.215
	Root	0.001	0.004
	LSD (0.05)	0.116	0.076
Low density 5th unit	^{13}C fed leaf	0.074	0.078
	^{13}C fed leaf petiole	0.041	0.053
	Other leaves	0.001	0.000
	Stem	0.002	0.000
	Other pods	0.002	0.004
	Pods in an unit	0.000	0.067
	Root	0.000	0.001
	LSD (0.05)	0.029	0.035
High density 1st unit	^{13}C fed leaf	0.249	0.094
	^{13}C fed leaf petiole	0.156	0.074
	Other leaves	0.000	0.000
	Stem	0.014	0.001
	Other pods	0.013	0.021
	Pods in an unit	0.032	0.343
	Root	0.005	0.003
	LSD (0.05)	0.085	0.123
High density 5th unit	^{13}C fed leaf	0.178	0.070
	^{13}C fed leaf petiole	0.117	0.066
	Other leaves	0.000	0.000
	Stem	0.000	0.000
	Other pods	0.007	0.005
	Pods in an unit	0.001	0.020
	Root	0.002	0.002
	LSD (0.05)	0.073	0.031

LSD: least significant difference. The experiment was conducted in 2004

4 Discussion

Increased light interception available in narrow row planting was proposed to generate more biomass in soybean plant (Board *et al.*, 1990). High vegetative biomass was considered beneficial for seed filling (Kane and Grabau, 1992). Responses of genotypes to light perception, however, were not identical because of differences in physiological factors associated with assimilate supply to reproductive sinks (Kumudini,

2002). The discovery made in our study supports this proposition and reveals for the first time that assimilate partitioning of soybean genotypes to reproductive organs is not identical at different population densities. The cultivar Enrei, which is widely used in Japan, failed to increase yield at a high population density. In contrast, the low yielding cultivar Shakujo responded well to increased density and out-yielded Enrei at a high density of 18.5 plants/m² (Table 3). It was found that the pod weight per plant decreased significantly as density increased in both cultivars (Table 3, Fig. 5). Under these constraints, the terminal location of the big raceme comprised of a few hundreds of pods in Shakujo proved more advantageous for ^{13}C partitioning, in comparison to Enrei having axillary racemes. In grain legumes like soybean (*Glycine max* L.) (Nobuyasu *et al.* 2003) and field bean (*Phaseolus vulgaris* L.) (Tanaka and Fujita, 1979), a source-sink unit was proposed to comprise a leaf, truss below the leaf node, and the axillary raceme containing several pods on it. This proposition matched partially for the Enrei cultivar in our study, but not so for Shakujo. In Enrei, the upper leaf exported ^{13}C assimilates to the axillary pods as well as pods above it. The translocation was poor from the lower leaf and it exported to the axillary pod only (Fig. 1). In Shakujo, the upper leaves supplied carbon assimilates to the apical pods and when they were removed, assimilates accumulated mostly in the fed leaf (data not shown). In addition to the first leaf, some other leaves on lower nodes also contributed significantly to the apical pods (Fig. 1). These findings corroborate the supposition addressed for rice (Mohapatra *et al.*, 2004) that a larger panicle in a high yielding variety is composed of a source-sink unit with remotely located lower leaves in addition to flag leaf, mainly due to stronger sink activity. Namely, source-sink unit varies depending upon the capacity of sink activity: the greater the sink activity, the wider the source leaf. Broadening of the source area seems to be an advantage for Shakujo for grain filling. Another advantage for grain production in this cultivar is that the source-sink unit is located on the top of the plant, which may avoid shading by their own leaves. However, under these conditions, these upper leaves are mutually shaded by leaves in the surrounding plants.

It has been reported that soybean racemes located on the lower part of the plant are frequently

shaded in field grown conditions (Fig. 7) (Ofosu-Budu *et al.*, 1990). In Enrei, the fifth leaf poorly assimilated the carbon isotope supplied to it (Fig. 9, Table 4). Similarly, the assimilation capacity of the leaf on the same node of Shakujo was also lower. The upper leaf of Enrei assimilated more carbon, but could not export it to the pods. In addition, the leaf area distribution at the lower strata of the canopy in Enrei increased when the plants were grown under high density of population and this effect was not evident in Shakujo (Fig. 4). Similar to leaf area, pod distribution increased in the lower strata of Enrei and decreased in the upper strata (Fig. 5). Since pod development on soybean is an indeterminate process, the older pods developed and filled properly at an early stage of plant development, when competition between plants was not severe at high planting density (Duncan, 1986). Pod weight of the uppermost stratum was reduced significantly because of intense competition between plants at later stage of development. It reduced assimilate partitioning from the upper leaf to the pods in Enrei (Fig. 5). Terminal location of pods and source leaves in Shakujo precluded this effect of planting density. Although yield per plant declined, the apical pods filled and ensured good yield at high density. High planting density could have reduced pod filling on the lower nodes of the Enrei cultivar, but yield contribution from these nodes was minimal (Table 1).

The gradient in pod weight between the upper and lower units was quite evident in Shakujo, but not in Enrei (Table 1). This gradient was equally matched by the differences in source capacities of the units individually in both the cultivars (Table 2). The lower leaves of Shakujo could not photosynthesize as much as that of the upper leaves. The increase of population density exacerbated the situation for the lower leaves, but not for the upper leaves (Figs. 7 and 8). This effect of population density could not be noted on the lower leaves of Enrei cultivar, but the spatial difference in rate of photosynthesis between the two types of leaves was similar to Shakujo. It has been reported that leaf carbon exchange capacity in soybean varies with time of origin, nodal position, developmental phase, genotypes, and environmental conditions (Dornhoff and Shibles, 1970; Abu-Shakra *et al.*, 1978; Gordon *et al.*, 1982; Egli, 1997). In our study, shading of the upper canopy layers and developmental stage might

have impacted leaf photosynthesis of the older leaves on the lower units in both cultivars. Although source activity of upper leaves was not affected by population density in Enrei, pod development was impacted by lack of tolerance to stress.

In soybean, provision of assimilate alone does not improve grain filling, unless partitioning to the sink increases substantially (Kumudini, 2002). Apart from assimilates, hormones, nutrients, vascular constraints, and sink capacity influence partitioning of assimilates from the source to sink (Wardlaw, 1990). Competition between individuals at high density in our study could have influenced any of the factors and reduced pod development in the apical parts of the Enrei cultivar. It was reported that plants grown in sparse stands experience greater red/far-red light ratio within the canopy compared to those in dense stands (Sanchez *et al.*, 1993), and high red/far-red light ratio increased partitioning of dry matter into branches (Board, 2000). Increased exposition of plant parts to the high level of far-red light within the canopy at high density could have impaired assimilates transport and grain yield per plant in our study for both varieties. The effect was more evident, however, in Enrei than in Shakujo (Fig. 9, Table 4), because assimilates could not go to branches in the former. Improved assimilate export could not sustain pod development in the upper parts of Enrei (Fig. 5). Shakujo was relatively less affected because of the terminal location of pods. Taking this evidence into account, it is concluded that Enrei is more versatile for adaptation to density stress than Shakujo. The physiological basis for this adaptation remains oblivious presently. Lack of this information, however, does not preclude Shakujo for consideration as an ideotype for soybean cultivation.

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